1742

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: §
J. M. Steinke §
A.P. Shepherd §
§
Serial No.: 07/953,680 §
§
Filed: September 29, 1992 §
§
For: METHOD AND APPARATUS §
FOR DIRECT SPECTROPHOTO- §
METRIC MEASUREMENTS IN §
UNALTERED WHOLE BLOOD §

Examiner: K. Hantis

Group Art Unit: 2505

#17½

Atty. Dkt.: UTSK:142/BAH

CERTIFICATE OF MAILING
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231, on the date below:

October 31, 1995 — David D. Bahler
Date **David D. Bahler**

DECLARATION OF A. P. SHEPHERD AND JOHN M. STEINKE UNDER 37 C.F.R. §1.131

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

A.P. SHEPHERD and JOHN M. STEINKE declare as follows:

1. We are the co-inventors of the subject matter disclosed and claimed in the above-identified U.S. Patent Application Serial No. 07/953,680.

2. We have reviewed the disclosure of U.S. Patent No. 5,064,282 to Curtis ("CURTIS"), having a filing date of September 26, 1989.

3. With respect to so much of the subject matter recited in claims 1-36 presently pending in the above-identified patent

application as is shown by CURTIS, we reduced that subject matter to practice in the United States well prior to September 26, 1989. The circumstances of the reduction to practice are presented in the following paragraphs.

4. Well prior to September 26, 1989, we conceived of a system for the direct measurement of multiple hemoglobin species in unaltered whole blood. In particular we conceived of and built a system for measuring four different hemoglobin species in whole blood: oxy-, deoxy-, carboxy-, and met-hemoglobin. That system included: the generation of four wavelengths (one for each hemoglobin species being measured) that had been selected to maximize absorbance relative to scattering; an extremely short cuvette-to-detector distance; a very thin sample cuvette; a large area light detector; a source of monochromatic light; and a computer for solving four simultaneous linear equations to compute the concentrations of each of the four species being measured.

5. Exhibit 1 is a Confidentiality Agreement, with attachment, signed by us and by George F. Sedivy of Waters Instruments, Inc., prior to September 26, 1989. The attachment to that Confidentiality Agreement is a disclosure of our invention as it then existed.

6. Substantially contemporaneous with the signing of the Confidentiality Agreement mentioned in the preceding paragraph, Mr. Sedivy visited our laboratory at the University of Texas Health Science Center in San Antonio, Texas and observed, well prior to September 26, 1989, a system in operation constructed in accordance with the attachment to the Confidentiality Agreement.

7. After the system for measuring the concentrations of hemoglobin species in unaltered whole blood was constructed, it was tested by us prior to September 26, 1989. Exhibit 2 hereto is a letter, with three attached graphs, to counsel for the University

of Texas, David D. Bahler, dated prior to September 26, 1989. A portion of that letter has been redacted to withhold attorney-client privileged communication. The attached graph entitled "Present Invention" depicts test results obtained using our system and shows how our system renders substantially linear the relationship between optical density and hemoglobin concentration. These test results demonstrated to our satisfaction that the system was capable of measuring the concentrations of hemoglobin species in unaltered whole blood. In addition, we conducted tests prior to September 26, 1989 using the system described in Exhibit 1, to show to our satisfaction that our system produced satisfactory measurements of the percentages of oxy-, carboxy-, deoxy-, and met-hemoglobin when compared with independent measurements.

8. The disclosure of our system included in Exhibit 1, and the graphs included in Exhibit 2 formed part of the basis for our U. S. Patent Application Serial No. 07\313,911, filed February 23, 1989. That patent application accurately depicted our system for the direct measurement of multiple hemoglobin species in unaltered whole blood as it existed on February 23, 1989.

9. Neither our system that existed prior to September 26, 1989, nor our Patent Application Serial No. 07\313,919 embodied or disclosed the use of a scattering subset of wavelengths used to correct the calculations of the concentrations of hemoglobin species for the effects of radiation scattering in unaltered whole blood. That disclosure is also not in the CURTIS reference. That feature is disclosed and claimed only in the present Patent Application Serial No. 07\953,680.

10. The construction and successful testing of the spectrophotometric apparatus of our invention prior to September 26, 1989, as reflected by the statements in this Declaration and the Exhibits appended hereto, occurred in the United States and constituted a reduction to practice of our invention, without the

generation of wavelengths for scattering correction, prior to September 26, 1989, the earliest date that CURTIS can be prior art.

11. We declare that all statements made herein of our own knowledge are true, and that all statements of our own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject patent application, and any patent issuing thereon.

4 Oct. 95
Date

A. P. Shepherd

4 October 1995
Date

John M. Steinke

h:\utsk\142\pto\13.bah

John M. Steinke

A.P. SHEPHERD, PH.D.
DEPARTMENT OF PHYSIOLOGY
U.T. HEALTH SCIENCE CENTER
7703 FLOYD CURL DRIVE
SAN ANTONIO, TEXAS 78284

CONFIDENTIALITY AGREEMENT

AGREEMENT and acknowledgement between _____ (THE INVENTOR) and WATERS INSTRUMENTS, INC., (THE COMPANY).

Whereas the INVENTOR agrees to furnish the COMPANY certain confidential information relating to the affairs of the INVENTOR for purposes of: (Describe)

> Developing an oximeter that uses whole blood to measure %HbO2, %Sat O2, %CO, and %Met, and is competitive with current products in accuracy and price.

Whereas, the COMPANY agrees to review, examine, inspect or obtain such information only for the purposes described above, and to otherwise hold such information confidential pursuant to the terms of this agreement.

BE IT KNOWN, that the INVENTOR has or shall furnish to the undersigned certain confidential information, as set forth on attached list, and may further allow the COMPANY the right to inspect the facilities of the INVENTOR and/or interview employees or co-workers of the INVENTOR, all on the following conditions:

1. The COMPANY agrees to hold all confidential or proprietary information or trade secrets ("information") in trust and confidence and agrees that it shall be used only for the contemplated purpose, shall not be used for any other purpose or disclosed to any third party.

2. The COMPANY agrees not to manufacture, nor cause to be manufactured, nor offer for sale, any product suggested by the confidential information for a period of ten (10) years provided the confidential information does not become public knowledge prior to an agreement in which the COMPANY and the INVENTOR mutually agree to declare this Confidentiality Agreement null and void.

3. No copies will be made or retained of any written information supplied without permission from the inventor.

4. At the conclusion of our discussions, or upon demand by the inventor, all information, including written notes, photographs, memoranda, or notes taken shall be returned.

5. This information shall not be disclosed to any employee or consultant unless they agree to execute and be bound by the terms of this agreement.

6. It is understood that the COMPANY shall have no obligation with respect to any information known by the COMPANY or generally known within the industry prior to date of this agreement, or becomes common knowledge within the industry thereafter.

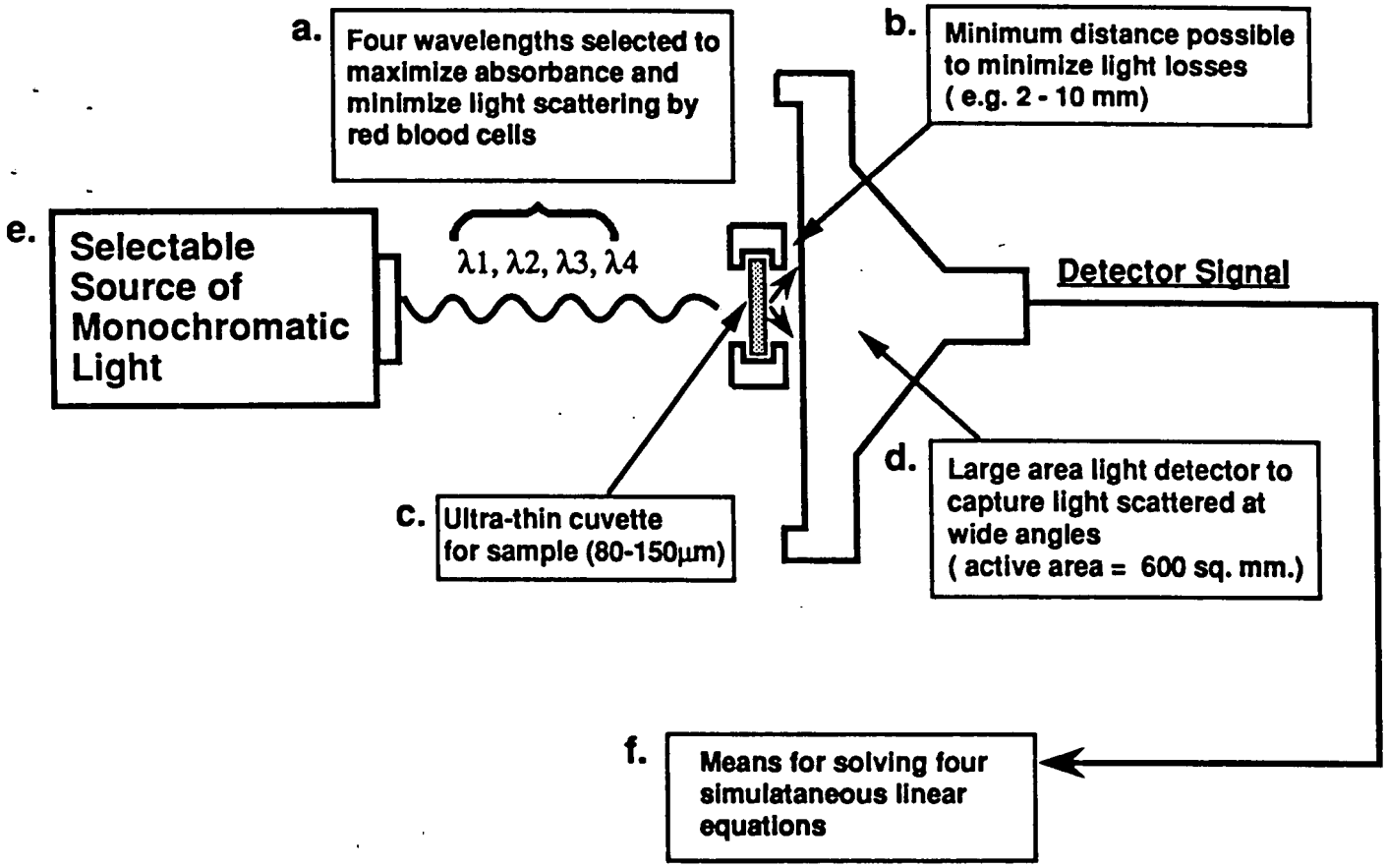
Dated: _____

A.P. Shepherd John M. Steinke
(THE INVENTOR)

George F Selwig
WATERS INSTRUMENTS, INC.

Invention: Direct Measurement of Multiple Hemoglobin Species in Whole Blood

Inventors: A.P. Shepherd and J. M. Steinke



Purpose of the Invention

The invention is a spectrophotometric means of measuring the concentrations of as many as four different species of hemoglobin in whole blood, e.g. oxy-, deoxy-, carboxy-, and met-hemoglobin. Unlike existing instruments, the new method measures the absorbance of light in whole, undiluted blood. The chief advantage of the method over prior art is that pumps and fluid systems to dilute and hemolyze the sample are not necessary, thus reducing the cost of manufacturing the new instrument.

Rationale of the Invention

Two processes attenuate the transmission of light in whole blood: optical absorbance and light scattering. Previous instruments that measure as many as four different species of hemoglobin hemolyze the blood sample and dilute it to eliminate the light scattering that the cells cause. The resulting hemoglobin solution thus obeys Beer's Law, and the hemoglobin species can be deduced by measuring the optical absorbance at four appropriate wavelengths.

By contrast, the present invention uses design features which maximize the optical absorbance of whole blood and minimize light scattering so that the apparent optical density of the sample is primarily due to absorbance with as small a contribution by light scattering as possible.

Details of the Invention

As the attached figure shows, the essential features of the invention consist of (a) four selected wavelengths, (b) an extremely short cuvette-to-detector distance, (c) a very thin sample cuvette, (d) a large area light detector, (e) a source of monochromatic light, and (f) a computer or means for solving four simultaneous linear equations:

a. Wavelengths: The wavelengths are selected from regions of hemoglobin's absorbance spectrum where absorbance is much greater than light scattering. They are also chosen to maximize the measurement accuracy in distinguishing one hemoglobin species from another and to minimize the $d\varepsilon/d\lambda$ values where possible to avoid variations of extinction coefficients (ε) induced by small variations in the wavelength (λ) emitted by the source.

b. Cuvette-to-Detector Distance: The cuvette-to-detector distance is made as short as possible to minimize light losses caused by light scattered at large angles. The smaller the cuvette-to-detector distance, the greater will be the receiving aperture half angle (α) of the detector.

c. Cuvette Light Path: An ultra-thin cuvette with a sample depth of 80-150 μm is used so that relatively few red blood cells are in the illuminated path compared to conventional cuvettes. This strategy minimizes total light scattering relative to absorbance by reducing the chance that a photon will be scattered by more than one red blood cell. These necessarily short pathlengths also insure that the optical density values are small enough to be conveniently measured, since wavelengths are chosen at which the extinction coefficients of hemoglobin are extremely large.

d. Detector. A light detector with a relatively large area such as 600 sq.mm. is used to capture light scattered at wide angles. More precisely, the receiving aperture half angle α of the detector should be made as large as possible (e.g. $\alpha > 70°$).

e. Light Source: In the preferred embodiment of the invention, several different light sources could possibly be used to select the four wavelengths: (1) a white light source and interference filters, (2) a tunable laser, (3) light-emitting diodes and interference filters, (4) a white light source in combination with a monochromator or (5) a white light source and a diffraction grating.

f. Computer or Means for Solving Four Simultaneous Linear Equations: In the preferred embodiment of the invention, the incident and transmitted light intensities (I) would rapidly and sequentially be measured at each wavelength to obtain the optical density OD = log (I_o/I). The optical design features outlined above make Beer's Law a reasonable approximation of the OD of whole blood. Therefore, the following equations relate the OD at each wavelength to the concentrations of each of the four hemoglobin species:

$$OD\lambda_1/D = \varepsilon_{11}*CHB + \varepsilon_{12}*CHBO2 + \varepsilon_{13}*CHBCO + \varepsilon_{14}*CHBMET \quad (1a)$$

$$OD\lambda_2/D = \varepsilon_{21}*CHB + \varepsilon_{22}*CHBO2 + \varepsilon_{23}*CHBCO + \varepsilon_{24}*CHBMET \quad (1b)$$

$$OD\lambda_3/D = \varepsilon_{31}*CHB + \varepsilon_{32}*CHBO2 + \varepsilon_{33}*CHBCO + \varepsilon_{34}*CHBMET \quad (1c)$$

$$OD\lambda_4/D = \varepsilon_{41}*CHB + \varepsilon_{42}*CHBO2 + \varepsilon_{43}*CHBCO + \varepsilon_{44}*CHBMET \quad (1d)$$

where $\varepsilon_{11}, \varepsilon_{12}, \ldots, \varepsilon_{44}$ are the appropriate extinction coefficients at the given wavelength and for the given hemoglobin species, and D is the depth of the sample. Once the four ODs are obtained at $\lambda_1, \lambda_2, \lambda_3$, and λ_4, the four equations above are easily solved for the four concentrations CHB, CHBO2, CHBCO and CHBMET. The individual saturations are then obtained from the ratios CHB/CTOT, CHBO2/CTOT, CHBCO/CTOT, and CHBMET/CTOT where CTOT = CHB + CHBO2 + CHBCO + CHBMET.

A computer, microprocessor, or other appropriate means could be used to solve this system of equations and display the result to the operator.

The University of Texas
Health Science Center at San Antonio
7703 Floyd Curl Drive
San Antonio, Texas 78284-7756

Department of Physiology (512) 567-4400

Mr. David Bahler
Arnold, White & Durkee
600 Congress Street
Austin, Texas 78701

Subject: UTSK:097

Dear Mr. Bahler:

In response to your request for material to "beef up" our Four Wavelength Hemoglobinometer-Oximeter, we are enclosing several items:

1. absorbance spectra for hemoglobin solutions. These spectra show the differences in the absorption of light by the four hemoglobin species of interest.

2. two graphs showing that for whole blood the relation between optical density and hemoglobin concentration is nonlinear in a conventional optical geometry, whereas the present invention renders the relation linear.

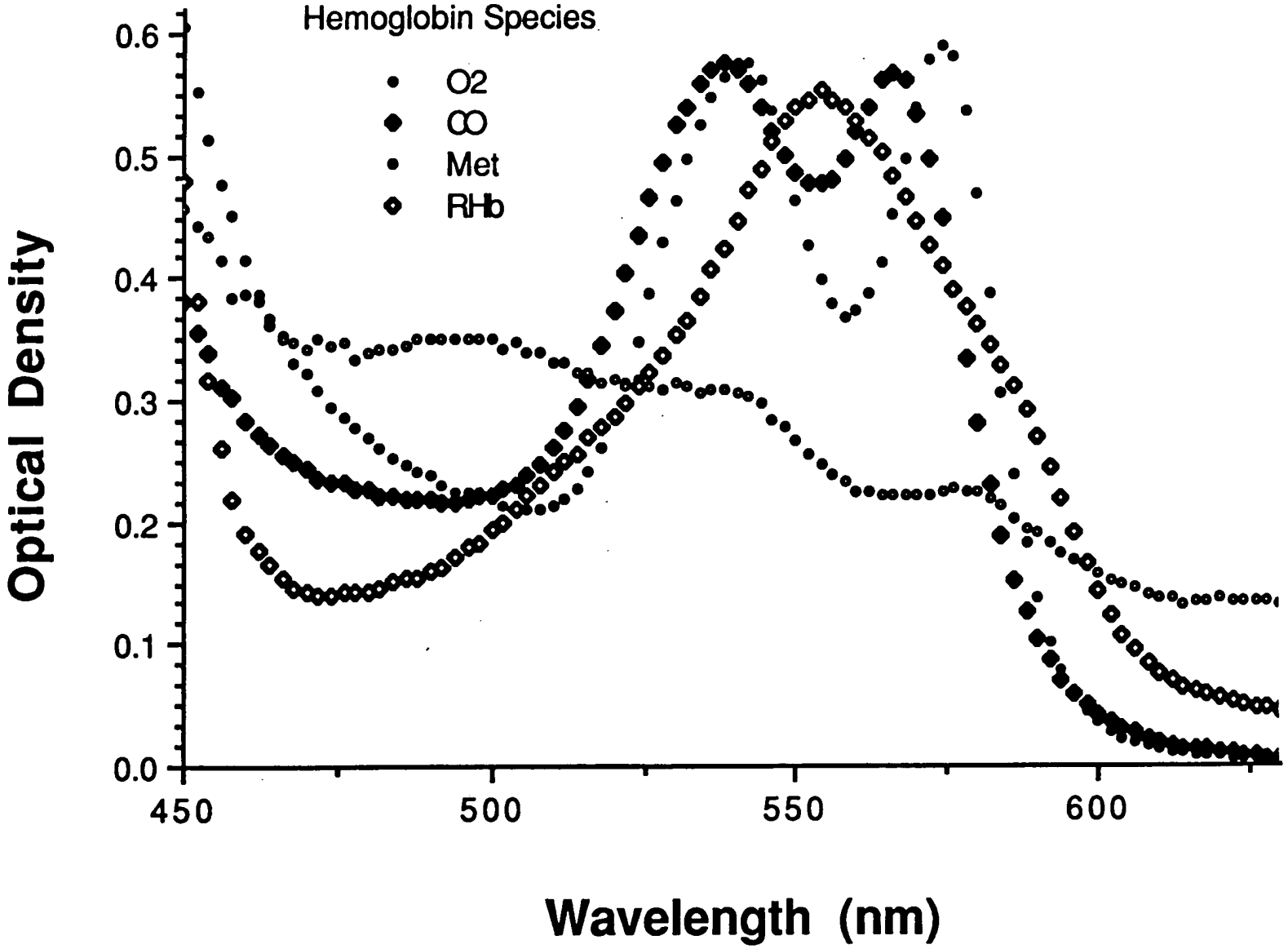
In addition to these figures, we can also send you graphs showing how well our method works when its measurements are compared with independent measurements of the percentages of oxy-, carboxy-, deoxy-, and met-hemoglobin. We don't have these yet, but we could certainly provide them to you before the application is filed.

If you can think of anything else that would strengthen our application, please let us know. Thank you for your help.

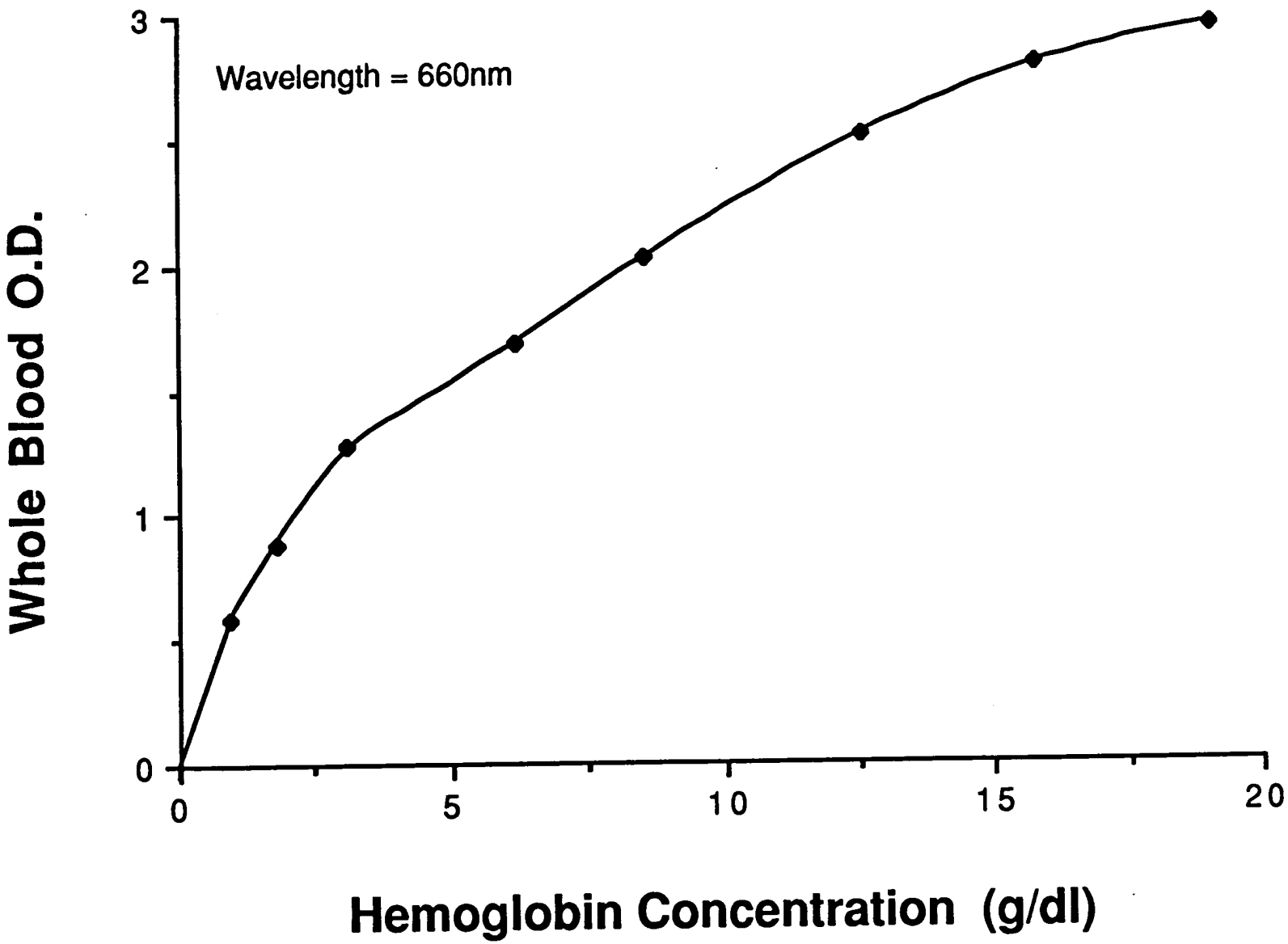
Sincerely,

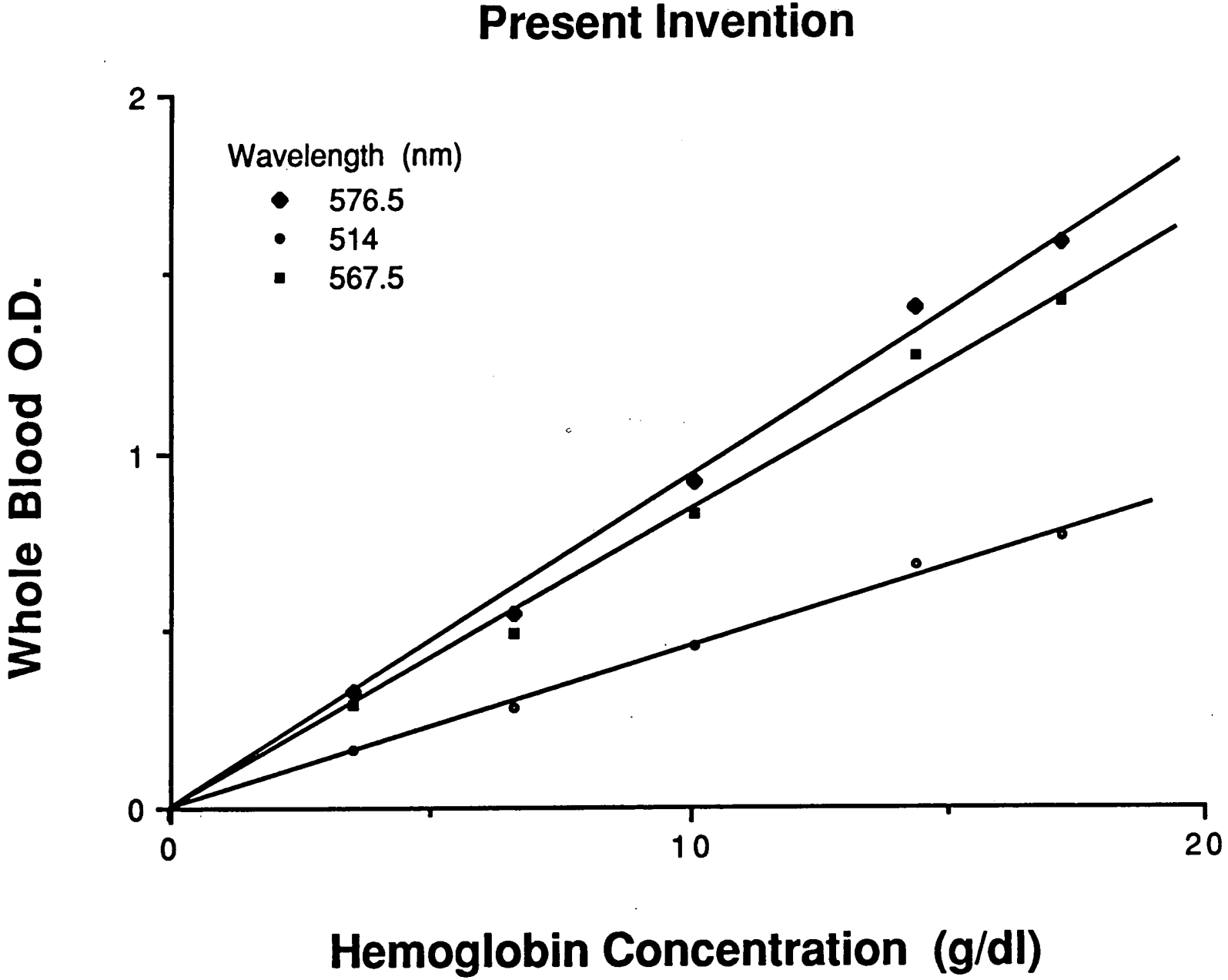
A. P. Shepherd, Ph.D.
Professor

Enclosures


Hemoglobin Species
O2
CO
Met
RHb
Optical Density
0.0
0.1
0.2
0.3
0.4
0.5
0.6
450
500
550
600
Wavelength (nm)

Conventional Optics




Present Invention
Wavelength (nm)
576.5
514
567.5
Whole Blood O.D.
2
1
0
0
10
20
Hemoglobin Concentration (g/dl)